

Relation Between Mitral Regurgitation and Platelet Activation

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Objectives. This study sought to examine the effect of mitral regurgitation (MR) on platelet activation in patients with mitral valve prolapse (MVP) or rheumatic MR.

Background. MVP and rheumatic MR are associated with an increased incidence of thromboembolic events. Although the underlying causes are not clear, increased platelet activation has been suggested as one of the pathogenic mechanisms. Results of previous studies that have investigated the relation between MVP and platelet activation are controversial. Whether the presence of MR in patients with mitral valve disease is associated with platelet activation remains unclear.

Methods. We studied platelet activation by measuring the plasma level of platelet factor 4 (PF4) and beta-thromboglobulin (BTG) in 16 patients with MVP, 12 patients with rheumatic MR and 25 control subjects. A detailed echocardiographic examination, including M-mode measurement and color Doppler flow mapping to detect the presence and severity of MR was performed.

Results. Patients and control subjects were matched for gender, age and left ventricular ejection fraction. Eight (50%) of 16 patients with MVP had MR. Patients with MVP and MR and patients with rheumatic MR had a significantly larger left atrial

diameter. Mean log plasma levels of PF4 and BTG were significantly higher in patients with MVP and MR and patients with rheumatic MR than in control subjects (1.17 ± 0.22 and 0.93 ± 0.23 IU/ml vs. 0.52 ± 0.34 IU/ml, $p < 0.01$; 1.70 ± 0.21 and 1.53 ± 0.15 IU/ml vs. 1.37 ± 0.15 IU/ml, $p < 0.05$, respectively) but were comparable in patients with MVP and no MR and control subjects. Plasma levels of PF4 and BTG were positively correlated with the severity of MR, as assessed by a semiquantitative method ($r = 0.59$, $p = 0.0001$; $r = 0.60$, $p = 0.0001$, respectively). Increasing age and left atrial enlargement were not related to platelet activation.

Conclusions. MR in mitral valve disease was associated with systemic platelet activation. MVP itself was not associated with increased platelet activation. The degree of platelet activation was positively correlated with the severity of MR and was independent of the underlying etiology of mitral valve disease, age and left atrial size. The possibility of a higher incidence of thromboembolism and the role of antiplatelet agents in such patients will require further studies to determine.

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Mitral valve prolapse (MVP) is the most common valvular heart disease and has a prevalence of 3% to 4% in the general population (1-5). Although the long-term prognosis of MVP is usually benign, thromboembolic complications, including cerebral and retinal ischemic events, may occur (6-11). Previous studies (6,8) have demonstrated a fourfold to sixfold higher incidence of MVP in young patients with cerebral ischemia than in age-matched control subjects. The etiology of increasing thromboembolic events in patients with MVP is unknown. It has been suggested (12) that these events may be related to platelet activation due to exposure to an abnormal valvular surface or hemodynamic irregularity. However, previous studies (13-19) that have investigated platelet activation in patients with MVP have yielded controversial results. The broad spectrum of patients with MVP probably explains the different results obtained in studies of platelet activation. Patients with MVP are heterogeneous with regard to left atrial size and the

presence or absence and severity of mitral regurgitation (MR). The thromboembolic risk of patients with rheumatic MR is also increased and is higher in those with severe MR (20,21). Whether MR in MVP may result in platelet activation and, subsequently, an increased risk of thromboembolism is unclear. The purpose of the present study was to determine the significance of MR on platelet activation by evaluating two markers of platelet activation in patients with MVP or rheumatic MR.

Methods

Study patients. The study included 16 patients (4 men, 12 women; mean $[\pm SD]$ age 44 ± 12 years) with echocardiographically documented MVP and 12 (4 men, 8 women; mean age 44 ± 12 years) with a previous history of acute rheumatic fever and echocardiographically documented rheumatic MR without significant mitral stenosis (mean mitral valve area 3.2 ± 0.3 cm²). These patients were consecutive outpatients seen at our cardiac clinic for asymptomatic cardiac murmur (18 [64%] of 28) or nonspecific complaints, such as palpitations or atypical chest pain (4 [36%] of 12). The control group included 25 subjects (10 men, 15 women; mean age 40 ± 11 years), all of whom had normal findings on physical examination, rest

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Abbreviations and Acronyms

ANOVA	= analysis of variance
BTG	= beta-thromboglobulin
ELISA	= enzyme-linked immunosorbent assay
MR	= mitral regurgitation
MVP	= mitral valve prolapse
PF4	= platelet factor 4
2D	= two-dimensional

electrocardiography and echocardiography. Neither patients nor control subjects had a history of coronary artery disease, cerebrovascular disease, peripheral vascular disease, diabetic mellitus, cancer, renal failure, deep vein thrombosis or pulmonary embolism, which are known to be associated with platelet activation (22). Furthermore, patients with previously documented severe MR, other coexisting valvular lesions or atrial tachyarrhythmia were also excluded. The study protocol was in agreement with the guidelines approved by the ethics committee of our local institution.

Echocardiographic study. Two-dimensional (2D), M-mode and color Doppler transthoracic echocardiographic studies were performed in all subjects (Acuson, model 128XP 10C, 2.5/3.5-MHz probe). The echocardiographic examinations were performed by an experienced technician and were reviewed in blinded manner by two physicians (H.-F.T., C.-P.L.). Two-dimensional parasternal long-axis, short-axis and apical four-chamber views were recorded to visualize the anterior and posterior mitral leaflets adequately. M-mode echocardiography was performed according to the recommendations of the American Society of Echocardiography (23). Mitral valve area was also measured by the pressure half-time method (24). Color Doppler was used to detect the presence of MR, and MR severity was assessed by a semiquantitative method. We measured the percent ratio of the maximal flow disturbance produced by the MR jet to the left atrial area and graded it according to the Nagle criteria (angiographic) as follows: 1) <20% was considered *mild*; 2) 20%–40% was graded as *moderate*; and 3) >40% was considered *severe* (24). MVP was diagnosed when the apposed mitral leaflets were displaced posterior to the C-D line ≥ 2 mm in late systole or ≥ 3 mm for holosystolic MVP on M-mode echocardiography. Holosystolic M-mode MVP was accepted only if confirmed by leaflet billowing into the left atrium on the parasternal or apical long-axis views (1,25–27). Rheumatic MR was diagnosed when the mitral leaflets were thickened and calcified and had evidence of fusion of mitral commissures or subvalvular apparatus on 2D examination and when MR was present on color Doppler (24,28).

Platelet activation study. Platelet activation was assessed by measuring both plasma levels of platelet factor 4 (PF4) and beta-thromboglobulin (BTG). Both are platelet-specific proteins secreted from the alpha-granules during the release reaction (22,29). These proteins can be accurately measured by enzyme-linked immunosorbent assay (ELISA), provided

that special precautions (see later) are taken to prevent in vitro activation during phlebotomy and plasma processing (29). We measured both PF4 and BTG levels to assess in vivo platelet activation. High levels of BTG in the presence of normal or only slightly elevated levels of PF4 strongly suggest in vivo release, whereas comparable elevations in both suggest in vitro release. The ratio of BTG to PF4 was used to exclude in vitro activation; a significant increase in ratio (>3) suggested in vivo activation (30,31). Blood results with evidence of in vitro platelet activation (BTG/PF4 ratio $<3:1$) were not included for analysis.

Patients and control subjects were instructed to abstain from any medication or alcohol for 10 days before blood sampling. All blood samples were taken in the morning for convenience in processing. Precautions were taken to avoid ex vivo activation of platelet. Blood samples were drawn by venipuncture using a 21-gauge indwelling butterfly needle without the use of a tourniquet. The first 2 ml of blood was discarded, and the next 4 to 5 ml of blood was collected into a Diatube H tube (Diagnostic Stago, France) that was immediately placed in an icewater bath for at least 15 min and then centrifuged at 2,500g for 30 min at 4°C. After centrifugation, approximately one-third the volume of the plasma supernatant was collected by placing the pipette tip in the middle region of the supernatant to avoid aspirating any light platelets found at the top surface of the plasma and platelets resting on the top surface of the cell layer. The platelet-poor plasma was stored at -20°C . PF4 and BTG assays were carried out within 1 month of collection of the sample.

PF4 and BTG assays. PF4 and BTG determinations were performed by ELISA using a commercially available kit (Asserachrom PF4 and BTG, Diagnostica Stago) according to the manufacturer's instructions. Specific rabbit anti-human PF4 and BTG antibodies and anti-PF4- and anti-BTG-peroxidase conjugates were used. PF4 and BTG concentrations were determined from a PF4 and BTG absorbency calibration curve. Both PF4 and BTG assays were run with the same positive controls each time for calibration, and the results were highly reproducible ($>95\%$). One-third of the patients' samples were run in duplicate and the results were again comparable.

Statistical methods. Results are expressed as mean value \pm SD. Plasma levels of BTG and PF4 are logarithmically distributed; thus, log-transformed data were used for analysis (29). Differences in continuous variables were analyzed by paired or unpaired Student *t* tests or analysis of variance, as appropriate, and between-group comparisons were performed by multiple Bonferroni tests. Correlation between variables was determined by linear regression, with severity of MR converted to an ordinal scale (1–3). A *p* value <0.05 was considered significant.

Results

Clinical characteristics and echocardiographic variables (Table 1). The study groups were matched for age, gender and left ventricular ejection fraction. Eight patients with MVP

Table 1. Clinical and Echocardiographic Variables

	Control Subjects (n = 25)	Patients With Mitral Valve Prolapse				Patients With Rheumatic MR			
		All (n = 16)	p Value	With MR (n = 8)	p Value	Without MR (n = 8)	p Value	MR (n = 12)	p Value
Age (yr)	40 ± 10.9	43.8 ± 11.9	0.274	47.0 ± 9.7	0.098	40.5 ± 13.6	0.851	44.4 ± 12.1	0.307
Men/women	10/15	4/12	0.501	2/6	0.678	2/6	0.678	4/8	0.99
LVEF (%)	60.2 ± 7.9	64.6 ± 8.0	0.086	65.5 ± 8.0	0.219	63.8 ± 8.5	0.143	63.0 ± 7.0	0.294
LA (mm)	29.1 ± 7.6	34.8 ± 7.9	0.021*	39.8 ± 8.0	0.034*	29.8 ± 3.6	0.119	35.1 ± 6.9	0.0189*
Severity of MR									
Mild	NA	3		3		NA		9	
Ratio (%)		14 ± 5		14 ± 5				12 ± 3	
Moderate	NA	5		5		NA		3	
Ratio (%)		32 ± 6		32 ± 6				29 ± 4	

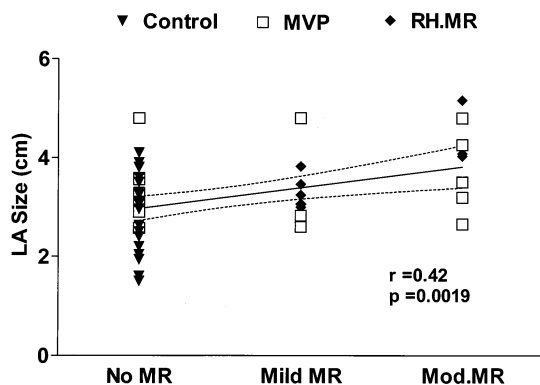
*p < 0.05 versus control subjects. Data presented are mean value ± SD, number of patients or regurgitant jet area/left atrial area ratio. LA = left atrium; LVEF = left ventricular ejection fraction; MR = mitral regurgitation; NA = not applicable.

(50%) and all patients with rheumatic MR (100%) had MR detected by color Doppler. Using the color Doppler semiquantitative method, three and five patients with MVP had mild and moderate MR, respectively, and nine and three patients with rheumatic MR had mild and moderate MR, respectively. In each subgroup of patients with mild or moderate MR, there was no significant difference in percent ratio of mitral regurgitant area/left atrial area between patients with MVP or rheumatic MR.

Left atrial diameter was significantly larger in patients with MVP and those with rheumatic MR than in control subjects. However, subgroup analysis showed that only patients with MVP with MR had left atrial enlargement. A modest but significant positive correlation was observed between left atrial diameter and severity of MR ($r = 0.42$, $p = 0.0019$) (Fig. 1).

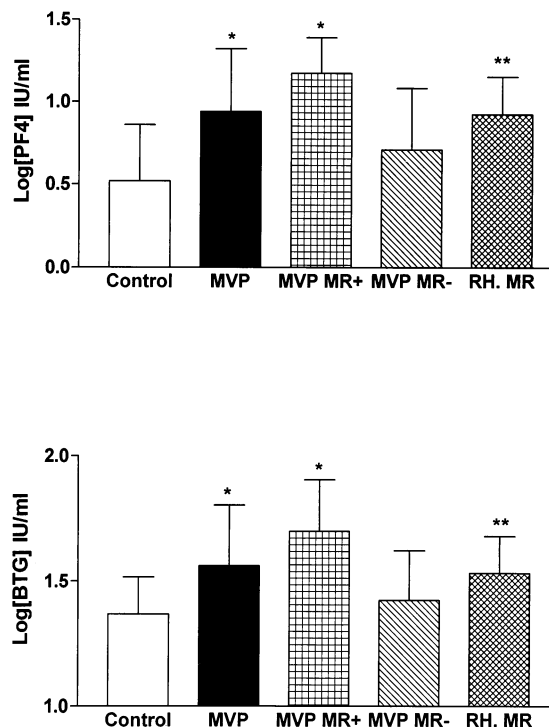
Platelet activation study. Mean log plasma levels of PF4 and BTG were significantly higher in patients with MVP and in those with rheumatic MR than in control subjects (0.94 ± 0.38 and 0.93 ± 0.23 IU/ml vs. 0.52 ± 0.34 IU/ml, $p < 0.01$; 1.56 ± 0.24 and 1.53 ± 0.15 IU/ml vs. 1.37 ± 0.15 IU/ml, $p < 0.05$, respectively) (Fig. 2).

Figure 1. Relation between left atrial (LA) size and severity of MR in patients with MVP or rheumatic MR (RH.MR) and control subjects. Solid line = fitted regression line; dotted lines = 95% confidence limits. Mod. = moderate.



Significance of MR in MVP. Subgroup analysis showed that plasma levels of PF4 and BTG were significantly increased only in patients with MVP with MR compared with control group values (1.17 ± 0.22 vs. 0.52 ± 0.34 IU/ml, $p < 0.001$; 1.70 ± 0.21 vs. 1.37 ± 0.15 IU/ml, $p < 0.001$, respectively). PF4 and BTG levels were comparable in patients with MVP without MR and control subjects. Furthermore, patients with MVP with MR also had significantly higher plasma levels of PF4 and BTG than those with MVP without MR (1.17 ± 0.22 vs. $0.71 \pm$

Figure 2. Log mean plasma levels of PF4 and BTG in patients with MVP or rheumatic MR (RH. MR) and control subjects. MR+ = with MR; MR- = without MR. Top, *p < 0.001, **p < 0.01 versus control subjects; bottom, *p < 0.01, **p < 0.05 versus control subjects.



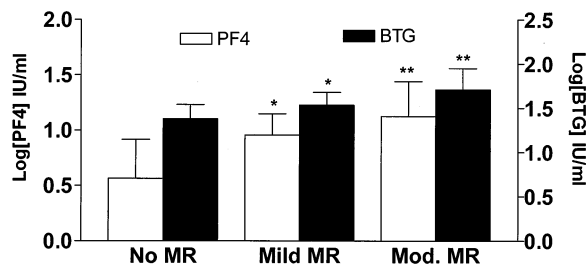


Figure 3. Log mean plasma levels of PF4 and BTG in patients with mild or moderate (Mod.) MR. * $p < 0.05$, ** $p < 0.001$ versus no MR.

0.37 IU/ml, $p < 0.05$; 1.70 ± 0.21 vs. 1.40 ± 0.20 IU/ml, $p < 0.01$, respectively) (Fig. 2).

Comparison between MVP and rheumatic MR. There was no significant difference in plasma levels of PF4 and BTG between patients with MVP and those with rheumatic MR. Patients with MVP with MR had significantly higher plasma levels of PF4 and BTG than patients with rheumatic MR (1.17 ± 0.22 vs. 0.93 ± 0.23 IU/ml, $p < 0.05$; 1.70 ± 0.21 vs. 1.53 ± 0.15 IU/ml, $p < 0.05$, respectively). However, after adjustment for severity of MR as a covariate, the difference in plasma levels of PF4 and BTG became insignificant ($p > 0.05$).

Relation between severity of MR and platelet activation. The presence of mild or moderate MR was associated with significantly increased plasma levels of PF4 and BTG (Fig. 3). Furthermore, a significant positive correlation was observed between severity of MR (none, mild or moderate) and plasma levels of PF4 and BTG ($r = 0.59$, $p = 0.0001$; $r = 0.60$, $p = 0.0001$, respectively) (Fig. 4).

Other variables and platelet activation. There was no age-dependence tendency ($r = 0.06$, $p = 0.68$; $r = 0.16$, $p = 0.26$, respectively) and only a weak correlation between left atrial diameter and plasma levels of PF4 and BTG ($r = 0.38$, $p = 0.005$; $r = 0.38$, $p = 0.005$, respectively).

Discussion

Background. Previous studies (5-11,32,33) evaluating the association between MVP and thromboembolism have yielded controversial results. Most patients with MVP have a benign course, but subsets of patients with clinical evidence of MR are at risk for development of complications (34-37). Patients with rheumatic MR have at least a medium risk of thromboembolism (38). The incidence of thromboembolism in these patients is ~1% to 3%/year and is higher in those with severe MR or associated mitral stenosis (20,21). Thus, the presence of MR may increase the risk of thromboembolic events in patients with MVP or rheumatic MR, although the underlying mechanism is still unclear.

Alterations in all phases of hemostasis are associated with thromboembolic events; however, platelet activation occurs in the earliest phase of arterial hemostasis and plays an important role in these disorders. Although a direct causal relation between platelet activation and thromboembolism is lacking, previous studies (39,40) have provided supportive evidence for

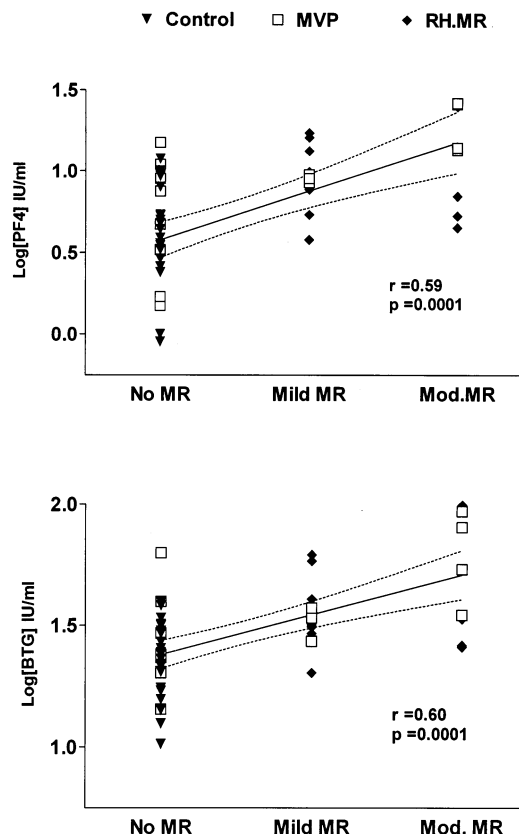


Figure 4. Relation between plasma levels of PF4 and BTG and severity of MR in patients with MVP or rheumatic MR (RH.MR) and control subjects. Mod. = moderate. Symbols as in Figure 1.

an association between them. Furthermore, the fact that antiplatelet agents are efficacious in the secondary prevention of thromboembolism provides further indirect evidence for this association (41). Previous studies (12,13,42) showed that abnormal platelet hyperactivity in patients with rheumatic mitral valve disease and MVP was associated with thromboembolism, leading to the hypothesis that platelet interaction with the irregular endocardial surface of the abnormal mitral valve might give rise to in vivo platelet activation, potentially resulting in thromboembolic events. However, previous studies (13-19) investigating platelet activation in MVP showed inconsistent results. The broad spectrum of subjects with MVP probably explains the different results obtained in studies of platelet activation. Patients with MVP are heterogeneous with regard to left atrial size, presence or absence of MR and the severity of MR. However, no study has investigated the relation between MR and platelet activation in patients with MVP or valvular heart disease.

Relation between MR and platelet activation in mitral valve disease. Both PF4 and BTG are specific platelet proteins that are stored in the alpha-granules and released in plasma during platelet activation. We found that patients with MVP and those with rheumatic MR had significantly higher plasma levels of PF4 and BTG than control subjects, and a subgroup analysis showed that patients with MVP with MR have signif-

icantly elevated plasma PF4 and BTG levels compared with those without MR and control subjects. MVP itself, without MR, was not associated with any significant difference in the levels of markers of platelet activation compared with control values. This finding may explain the discrepancy between previous studies on platelet activation in patients with MVP because the presence of MR was not considered in those studies. Both mild and moderate MR were associated with significant elevation in plasma levels of PF4 and BTG. More important, we demonstrated that severity of MR was positively correlated to degree of platelet activation. This finding is consistent with the clinical observation that patients with more severe MR had a higher incidence of thromboembolic events (21).

MVP with MR was also associated with significantly more elevated levels of PF4 and BTG than rheumatic MR and was related to the discrepancy in the proportion of patients with different grades of MR severity. The proportion of patients with moderate MR was higher in patients with MVP than in those MVP patients with rheumatic MR (63% vs. 25%). When severity of MR was also taken into consideration, valve disease etiology was not an independent predictor of increased platelet activation. Furthermore, the finding of a direct relation between degree of platelet activation and severity of MR also supports this observation. The control and study groups were matched for age and gender, and there was no association between the age and degree of platelet activation. As expected, the left atrium was larger in patients with MR, and there was a modest correlation between severity of MR and left atrial size. There was only a weak correlation between left atrial size and platelet activation, which may be related indirectly to MR.

Possible mechanism of platelet activation in MR. In patients with MVP, the formation of an adherent thrombus at the cul-de-sac created between the ballooning posterior leaflet and the atrial wall is usually attributed to a localized jet of MR (43,44). On cine ventriculograms during diastole, the regurgitant material is observed to swirl briefly into the left atrium and collect into a posteroinferior cul-de-sac, remaining stagnant there until the end of diastole (32). Thus, the mechanism of platelet activation associated with MR may be due to hemodynamic irregularities (turbulent flow in the left atrium) caused by the regurgitant jet in the presence of an abnormal valvular surface, independent of the underlying etiology. The activated platelet may then adhere and aggregate on the abnormal mitral valvular surface to form a platelet-fibrin thrombus on the leaflet, potentially resulting in thromboembolic events.

Clinical implications. The present study demonstrated that MR in patients with MVP or rheumatic MR was associated with systemic platelet activation. MVP itself was not associated with increased platelet activation. The degree of platelet activation was positively correlated with severity of MR and was independent of the underlying etiology of the mitral valve disease, age and left atrial size. Hence, both the presence and severity of MR are important factors that lead to platelet activation and may be an important pathogenic mech-

anism for the formation of platelet-fibrin thrombi on the abnormal mitral leaflet, subsequently leading to increased risk of thromboembolic events in patients with MVP or rheumatic MR.

Study limitations. Because all our patients had no previous history of thromboembolic events, we could not demonstrate a causal relation between platelet activation and thromboembolic events. The interpretation of PF4 and BTG results is often confounded by methodologic problems due to in vitro activation of platelet. However, special precautions were taken to reduce in vitro activation during the procedure, and the results were checked for evidence of in vitro activation, as previously described (29,30).

Conclusions. The present study demonstrated that in the presence of an abnormal mitral valve surface, as in MVP and rheumatic heart disease, even mild to moderate MR is associated with platelet activation. Whether these patients have a higher incidence of thromboembolism and what role antiplatelet agents play in such patients will require further studies.

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